IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of

U. Sanz
20 May, 2009

Ana Isabel SANZ MOLINERO Atty. Ref.: **4982-5**

Serial No. **10/537,897** Group: **1638**

Filed: **June 7, 2005** Examiner: **Baum**

For: **PLANTS HAVING MODIFIED GROWTH CHARACTERISTICS AND A METHOD FOR MAKING THE SAME**

* * * * * * * * * * *

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

RULE 132 DECLARATION

I, Ana Isabel Sanz Molinero, do hereby declare and say as follows:

1. I have reviewed the above-identified application, including the claims. I have also considered the documents listed herein. I am the inventor of the subject matter claimed in the above-identified application.

2. I copy of my Curriculum Vitae is attached.

3. I am familiar with the evidence submitted to the U.S. Patent Office in the above on November 18, 2008. I have been advised by the assignee's U.S. representative that the U.S. Patent Office representative examining the above has invited the applicants to present the evidence in the form of a Declaration. The present paper is being filed in response to this invitation.

4. I have produced the following evidence or had the evidence produced under my direction.

5. Example A - AtSTZparalog1: SEQ ID NO: 26 under the control of constitutive promoter GOS2

A DNA fragment encoding a 2XC2H2 protein represented in the application as filed by SEQ ID NO: 27 was isolated from an *Arabidopsis thaliana* seedling cDNA library (Invitrogen, Paisley, UK) by PCR amplification and subsequent cloning in an entry clone vector according to the methods described in Example 1 of the present application. SEQ ID NO: 27 polypeptide was encoded by the longest open reading frame of SEQ ID NO: 26 (AtSTZparalog1).

The primers used for the PCR amplification were as follows:

Forward primer: Ggggacaagtttgtacaaaaaagcaggcttaaacaatggccctcgaagcg
Reverse primer: Ggggaccactttgtacaagaaagctgggttcgagtattagatttttaaagataaatc

The entry clone was subsequently used in an LR reaction with a destination vector used for rice transformation to generate the plant expression vector pGOS2::AtSTZparalog1. The constitutive promoter, GOS2, was mentioned in Table 10 on page 48 of the application as filed as being a promoter useful in the methods of the invention.

Phenotypic characterization of the transformed plants was carried out essentially as described in Example 3 of the present application. The results are shown in Table I below.

Table I: Results of phenotypic characterization of T2 rice plants transformed with pGOS2::AtSTZparalog1.

pGOS2::AtSTZparalog1	
Parameter	% increase in the transgenic plants compared to the nullyzygous plants

pGOS2::AtSTZparalog1	
Parameter	% increase in the transgenic plants compared to the nullyzygous plants
Aboveground area	10
Root Area	4
Total Seed Weight	48
Number of filled seeds	46
Total number of seeds	12
Seed filing rate	30
Flowers per panicle	8
Harvest index	35
Days to flowering	4

The above results show that overexpression of the nucleic acid represented by SEQ ID NO: 27 (encoding the 2XC2H2 zinc finger protein represented by SEQ ID NO 26) under the control of a constitutive promoter (GOS2) gives:

Increased plant yield (in the form of increased root area, total seed weight, total number of seeds, number of filled seeds, seed filling rate, flowers per panicle and harvest index);

Increased leaf surface area (as manifested by increased aboveground area); and

Prolonged vegetative growth (where the time to flower was on average 4% longer in transgenic plants compared to corresponding nullyzygous plants).

6. Example B - AtSTZparalog1: SEQ ID NO: 26 under the control of seed-specific promoter prolamin

Ana-Isabel SANZ MOLINERO
Application No. 10/537,397
Rule 132 DECLARATION

A DNA fragment encoding a 2XC2H2 protein represented by SEQ ID NO: 27 was isolated from an *Arabidopsis thaliana* seedling cDNA library (Invitrogen, Paisley, UK) by PCR amplification and subsequent cloning in an entry clone vector according to the methods described in Example 1 of the present application. SEQ ID NO: 27 polypeptide was encoded by the longest open reading frame of SEQ ID NO: 26 (AtSTZparalog1).

The primers used for the PCR amplification were as follows:

Forward primer: Ggggacaagtttgtacaaaaaagcaggcttaaacaatggccctcgaagcg
Reverse primer: Ggggaccactttgtacaagaaagctgggttcgagtattagatttttaaagataaatc

The entry clone was subsequently used in an LR reaction with a destination vectors used for rice transformation to generate the plant expression vector pPROLAMIN::AtSTZparalog1. The seed-specific promoter, prolamin, was mentioned in Table 10 on page 48 of the application as filed as being a promoter useful in the methods of the invention.

Phenotypic characterization of the transformed plants was carried out essentially as described in Example 3 of the present application. The results are shown in Table II below.

Table II: Results of phenotypic characterization of T2 rice plants transformed with pPROLAMIN::AtSTZparalog1.

	pPROLAMIN::AtSTZparalog1
Parameter	% increase in the transgenic plants compared to the nullyzygous plants
Aboveground area	4
Total Seed Weight	8

pPROLAMIN::AtSTZparalog1	
Parameter	% increase in the transgenic plants compared to the nullyzygous plants
Number of filled seeds	7
Total number of seeds	5
Flowers per panicle	4
Harvest index	5

The above results show that overexpression of the nucleic acid represented by SEQ ID NO: 27 (encoding the 2XC2H2 zinc finger protein represented by SEQ ID NO 26) under the control of a seed-specific promoter (prolamin) gives:

Increased plant yield (in the form of increased aboveground area, total seed weight, total number of seeds, number of filled seeds, flowers per panicle and harvest index); and

Increased leaf surface area (as manifested by increased aboveground area).

7. Example C - OsSTZ(ortholog): SEQ ID NO: 36 under the control of root-specific promoter RCc3

A DNA fragment comprising the coding region of SEQ ID NO 36 was PCR amplified and cloned using methods essentially as described in the Examples section of the present application.

The primers used for the PCR amplification were as follows:

Forward primer: ggggacaagtttgtacaaaaaagcaggcttaaacaatgtcgagcgcgtcgt
Reverse primer: ggggaccactttgtacaagaaagctgggtctgaattacgcggtgagaag

Ana Isabel SANZ MOLINERO
Application No. 10/537,397
Rule 132 DECLARATION

A plant transformation vector carrying the coding region of SEQ ID NO 36 under the control of the root specific promoter, RCc3, was made, giving construct CD10315 described in Table 9 on page 47 of the present application. Agrobaterium-mediated transformation of rice plants was carried out to generate transgenic rice plants carrying the construct CD10315.

The results of the phenotypic evaluation of the CD10315-transgenic plants of the T2 generation are shown in Table III below.

Table III: Results of phenotypic characterization of T2 rice plants transformed with pRCc3::OsSTZorthblog.

	CD10315 plants
Parameter	% increase in the transgenic plants compared to the nullyzygous plants
Aboveground area	6
Root Area	5
Total Seed Weight	29
Number of filled seeds	29
Seed filling rate	16
Total number of seeds	17
Harvest index	25

The above results show that overexpression of a nucleic acid encoding the 2XC2H2 zinc finger protein represented by SEQ ID NO 36) under the control of a root-specific promoter (RCc3) gives:

Ann Isabel SANZ MOLINERO
Application No. 10/537,397
Rule 132 DECLARATION

Increased plant yield (in the form of increased aboveground area, root area, total seed weight, total number of seeds, number of filled seeds, seed filling rate and harvest index); and

Increased leaf surface area (as manifested by increased aboveground area).

8. Example D – AtSTZparalog2: SEQ ID NO:28 under the control of the beta-expansine EXPB9 (PRO061 2)

Ann Isabel SANZ MOLINERO
Application No. 10/537,997

Rule 132 DECLARATION

A DNA fragment encoding a 2XC2H2 protein represented in the application as filed by SEQ ID NO: 29 was isolated from an *Arabidopsis thaliana* seedling cDNA library (Invitrogen, Paisley, UK) by PCR amplification and subsequent cloning in an entry clone vector according to the methods described in Example 1 of the present application. SEQ ID NO: 29 polypeptide was encoded by the longest open reading frame of SEQ ID NO: 28 (AtSTZparalog2).

The primers used for the PCR amplification were as follows:
Forward primer:

ggggacaagtttgtacaaaaaagcaggcttaaacaatggcacttgaaactcttact

Reverse primer:

ggggaccactttgtacaagaaagctgggtttcctaggtttatgtttaggg

The entry clone was subsequently used in an LR reaction with a destination vector used for rice transformation to generate the plant expression vector pEXPB9::AtSTZparalog2. The PCR amplified DNA fragment was operably linked to the promoter PROO61_2 (pEXPB9: beta-expansine EXPB9), which is described in Table 10 in Table 10 on page 48 of the application as filed

Phenotypic characterization of the transformed plants was carried out essentially as described in Example 3 of the present application. The results are shown in Table I below.

Table I: Results of phenotypic characterization of T1 rice plants transformed with pEXPB9::AtSTZparalog2.

	pEXPB9::AtSTZparalog2
Parameter	**% increase in the transgenic plants compared to the nullyzygous plants**
Number of filled seeds	6.2
Seed filling rate	3.4

Ana Isabel SANZ MOLINERO
Application No. 10/537,897
Rule 132 DECLARATION

Harvest index	3.9
Number of panicles	3.8

The above results show that overexpression of the nucleic acid represented by SEQ ID NO: 28 (encoding the 2XC2H2 zinc finger protein represented by SEQ ID NO 29) under the control of a promoter expressed in young expanding tissues (pEXP9) gives an increase in plant yield a shown by the increased in the number of filled seeds, the increase in the seed filling rate (proportion of filled seed with respect to the total number of seeds harvested per plant, increased in the harvest index and the increase in the number of panicles per plant.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Signed this 20 day of May, 2009.

(Signature) A. I. Sanz

(print name) Ana Isabel Sanz Molinero